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Fragile X Founder Effects and New Mutations in Finland

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The apparent associations between fragile X mutations and nearby microsatellites may reflect both founder effects and microsatellite instability. To gain further insight into their relative contributions, we typed a sample of 56 unrelated control and 37 fragile X chromosomes from an eastern Finnish population for *FMR1* CGG repeat lengths, AGG interspersion patterns, *DXS548*, *FRAXAC1*, FRAXE and a new polymorphic locus, *Alu-L*. In the controls, the most common *FMR1* allele was 30 repeats with a range of 20 to 47 and a calculated heterozygosity of 88%. A strong founder effect was observed for locus *DXS548* with 95% of fragile X chromosomes having the 21 CA repeat (196 bp) allele compared to 17% of controls, while none of the fragile X but 69% of controls had the 20 repeat allele. Although the *FRAXAC1* locus is much closer than *DXS548* to *FMR1* (7 kb vs. 150 kb), there was no significant difference between fragile X and control *FRAXAC1* allele distributions. The FRAXE repeat, located 600 kb distal to *FMR1*, was found to show strong linkage disequilibrium as well. A newly defined polymorphism, *Alu-L*, located at ~40 kb distal to the *FMR1* repeat, showed very low polymorphism in the Finnish samples. Analysis of the combined loci *DXS548-FRAXAC1-*FRAXE showed three founder haplotypes. Haplotype 21-19-16 was found on 27 (75%) of fragile X chromosomes but on none of controls. Three (8.4%) fragile X chromosomes had haplotypes 21-19-15, 21-19-20, and 21-19-25 differing from the common fragile X haplotype only in FRAXE. These could have arisen by recombination or from mutations of FRAXE. A second haplotype 21-18-17 was found in four (11.1%) fragile X chromosomes but only one (1.9%) control. This may represent a more recent founder mutation. A third haplotype 25-21-15, seen in two fragile X chromosomes (5.6%) and one (1.9%) control, was even less common and thus may represent an even more recent mutation or admixture of immigrant types. Analysis of the AGG interspersions within the *FMR1* CGG repeat showed that 7/8 premutation chromosomes lacked an AGG whereas all controls had at least one AGG. This supports the hypothesis that the mutation of AGG to CGG leads to repeat instability and mutational expansion. © 1996 Wiley-Liss, Inc.

KEY WORDS: fragile X syndrome, founder effects, microsatellites, FRAXE, Finland

INTRODUCTION

Fragile X syndrome is the most common inherited form of mental retardation [Brown and Jenkins, 1992]. The molecular basis of the fragile X mutation is usually an expansion of CGG repeats located in the 5′ exon of the *FMR1* gene [Verkerk et al., 1991]. Controls usually have <55 repeats, premutations 56-200, and full mutations >200 [Fu et al., 1991; Brown et al., 1993; Snow et al., 1993]. The expansion to >200 is associated with hypermethylation of the *FMR1* CpG island promotor region which silences gene expression [Pieretti, 1991]. A single point mutation ($367^{\text{Ile}\rightarrow\text{Asn}}$) involving a KH RNA binding domain of *FMR1* [De Boulle et al., 1993] and rare partial or complete deletions of *FMR1* [De Graaff et al., 1995; Gedeon et al., 1992; Hirst et al., 1995; Meijer et al., 1994; Trottier et al., 1994; Wöhrle et al., 1992] are also associated with the affected phenotype. Within the CGG repeat region are occasional interruptions with single AGGs leading to a high de-

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gree of internal polymorphism as now demonstrated by several studies [Eichler et al., 1994; Hirst et al., 1994; Kunst and Warren, 1994; Snow et al., 1994; Zhong et al., 1995]. These studies have shown that instability appears to be directly associated with the presence within the 3′ region of a long pure CGG repeat.

The underlying mutation mechanism which results in triplet expansion is still largely unknown. Two possible mechanisms for the CGG dynamic expansion were proposed [Richards and Sutherland, 1992a]. One involves the mutation of an interrupting AGG to a CGG and subsequent DNA slippage. The other postulates the presence of a secondary flanking locus which causes the expansion. Both mechanisms are based on ideas related to the presence of a founder chromosome effect [Richards et al., 1992b]. Studies, focused on fragile X founder effects, have typed either a single flanking microsatellite marker: *FRAXAC1* [Hirst et al., 1993] or *DXS548* [Buyle et al., 1993; Smits et al., 1993] or haplotypes of two markers: *FRAXAC1* and *FRAXAC2* [Arinami et al., 1993; Richards et al., 1992b,1994] or *DXS548* and *FRAXAC2* [Oudet et al., 1993a, b; Haataja et al., 1994; Malmgren et al., 1994] or three markers including *FRAXAC1*, *FRAXAC2*, and *DXS548* [Macpherson et al. 1994]. We found *FRAXAC2*, an *Alu*-associated polymorphism located in the second intron of *FMR1*, contained three polymorphic subregions of form (GT)x-(AT)y-(T)z whose accurate typing can only be determined by sequencing, was mutable, and did not demonstrate linkage disequilibrium with *FMR1* CGG repeats in a New York sampling [Zhong et al., 1993]. Therefore, we believe that using *FRAXAC2* as a marker for disequilibrium analysis may not be reliable [Zhong et al., 1993, 1994a]. Instead, we analyzed haplotypes of *FRAXAC1* with *DXS548* in Caucasian [Zhong et al., 1994b] and Chinese populations [Zhong et al., 1994c]. We found increased microsatellite heterogeneity, reflected by an increased frequency of rare haplotypes associated with the fragile mutation. This led us to suggest the fragile X mutation may promote microsatellite instability through gene conversion or by some more direct role [Zhong et al., 1994a].

To gain further insights into founder effects in fragile X, we undertook to analyze a set of fragile X related microsatellite polymorphisms (*DXS548, FRAXAC1*, FRAXE, and *Alu-L*) among a Finnish population. Studies of Finland have been useful for genetic founder studies as it represents a genetic isolate that was settled an estimated 100 generations ago [Norio et al., 1973]. Such studies have shown very strong founder effects for many genetic diseases such as cystic fibrosis [Kere et al., 1989], Huntington disease [Ikonen et al., 1992], and diastrophic dysplasia [Hästbacka et al., 1992]. In agreement with previous findings, we confirmed that there appear to be strong Finnish founder effects for fragile X [Oudet et al., 1993b; Haataja et al., 1994]. Our results support the hypothesis of a common original founding mutation which has become associated with differing haplotypes of a nearby repeat, FRAXE by mutation or recombinations. Two less common fragile X mutations with differing haplotypes appear to be present in the population as well.

MATERIALS AND METHODS

A total of 93 X chromosomes from eastern Finland (56 normal and 37 fragile X) that had previously been tested for fragile X by direct Southern blotting using StB12.3 [Ryynanen et al., 1995] were analyzed for *FRAXAC1*, *DXS548* [Zhong et al., 1993, 1994b], and FRAXE [Knight et al., 1993; Zhong et al., 1996a] as well as *FMR1* CGG repeat lengths by PCR [Brown et al., 1993]. A new single strand conformation polymorphism (SSCP) of the *FMR1* associated *Alu-L* (GenBank number L29074, beginning nucleotide 60002) (N. Zhong, unpublished data) was analyzed. The individuals tested were all unrelated except by marriage and had not been included in previous analysis of founder effects [Oudet et al., 1993b; Haataja et al., 1994]. All premutation chromosomes analyzed were from members of known fragile X families. Analysis of AGG interruptions within the CGG repeat was also carried out using our previously described method using PCR amplification, partial digestion with restriction enzyme, Mnl I, and probing with a 5′ end-labeled oligonucleotide [Zhong et al., 1995, 1996b]. This method does not detect other possible triplets, such as TGG or GGG but such variants have been only rarely observed by direct sequencing approaches [Hirst et al., 1994; Kunst and Warren, 1994; Snow et al., 1994].

RESULTS

FMR1 CGG Repeat Distribution in Finnish Controls

Among 56 unrelated control X chromosomes, there was a total number of 14 different CGG repeat length alleles. The most common repeat allele was 30 (23.2%) with a second mode of 23 repeats (14.3%) and a range of 20 to 47 repeats as illustrated in Figure 1. Of note was the finding of two peaks in the "gray zone" with four chromosomes (7.1%) having 47 repeats and three (5.4%) having 38 repeats. The overall heterozygosity ($1-\Sigma q^2$) for control lengths was 88%.

DXS548 Allele Frequencies in Finnish Controls and Fragile X Chromosomes

A marked difference was noted in the distribution of *DXS548* alleles among the Finnish control and fragile X chromosomes. There were only two alleles among the fragile X while there were five alleles among the controls, with 95% of the fragile X being the 21 CA repeat allele (196 bp) compared to 17% of the controls, ($P<<0.001$). Table I compares *DXS548* allele frequencies in three Caucasian control populations (New York, French and Belgian-Dutch) to the Finnish controls. Sixty-nine percent of Finnish control alleles were 20 repeats, similar to the controls in the three other populations, while none of the Finish fragile X chromosomes had this allele. Among the other Caucasian studies, approximately twice as many fragile X (21–30%) as controls (10–14%) have the 21 repeat allele where as in the Finnish sample the ratio of 5.6:1 is much higher (95%:17%). Our data for this marker are also in close agreement with two other previous studies on the Finnish [Oudet et al., 1993b; Haataja et al., 1994].

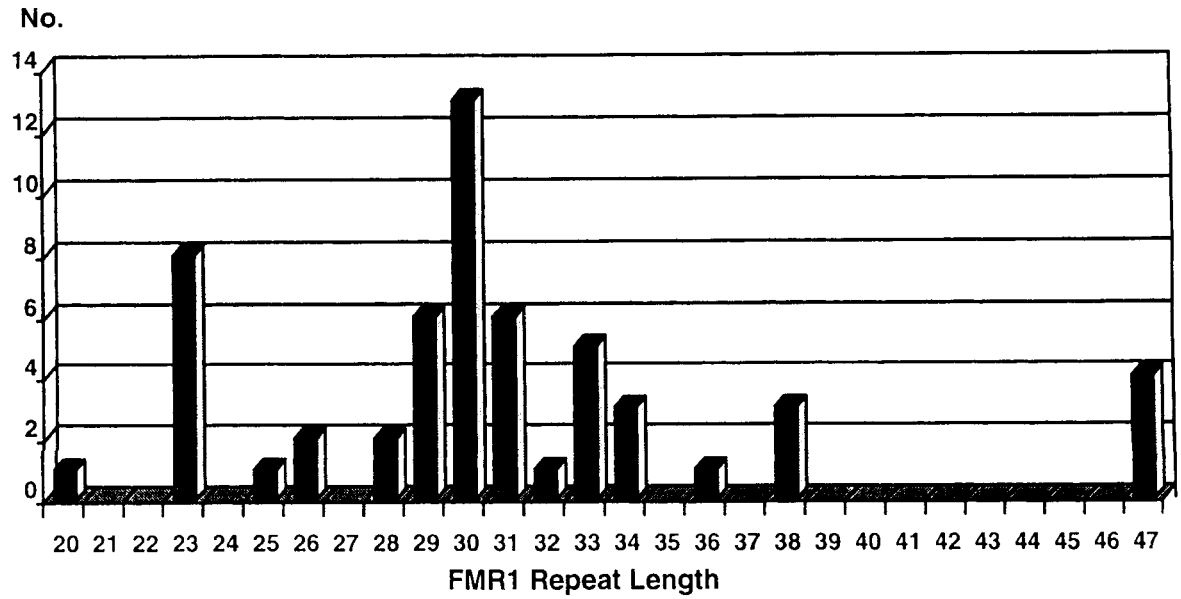

Fig. 1. Distribution of *FMR1* alleles in 56 Finnish controls.

These results provide strong evidence of a founder fragile X mutation in the Finnish population.

FRAXAC1 Allele Frequencies in Finnish Controls and Fragile X Chromosomes

The most common *FRAXAC1* repeat allele was 19 CA repeats, as indicated in Table II. This allele was present in 84% of the Finnish fragile X chromosomes, but was only present in 34–38% in the other Caucasian fragile X samples, a significant difference. However, this allele showed a non-significant difference between the fragile X (84%) and controls (61%) in the Finnish sample.

FRAXE Allele Frequencies in Finnish Controls and Fragile X Chromosomes

The triplet repeat, FRAXE, which is distal to the *FMR1* CGG repeat by 600 kb, had 13 different allele sizes ranging from 7 to 25 GCC repeats among the 56 control X chromosomes and 36 fragile X Finnish chromosomes, as summarized in Table III. The heterozygosity was 82% in the controls but only 42% in the fragile X. The mode allele of 16 FRAXE repeats was found of 75% of fragile X chromosomes compared to 30% of the controls ($P \ll 0.001$). Such differences were not seen in New York Caucasian chromosomes (25% vs. 27%, fragile X vs. controls) [Zhong et al., 1996a].

DXS548-FRAXAC1-FRAXE Haplotypes

Analysis of the three microsatellites (*DXS548*, *FRAXAC1* and FRAXE) together showed there were a total of 29 different haplotypes in the combined sample with 26 in controls and 6 in fragile X chromosomes, as indicated in Table IV. Three alleles (21-19-16, 21-19-15 and 21-19-20) differing only in FRAXE type, which were not found in any of the controls, accounted for 75%, 2.8% and 2.8% respectively of the fragile X chro-

TABLE I. Allele Frequencies of *DXS548* in Four Populations: Controls vs. FraX*

	Control series (n)				Fragile X series (n)			
CA repeats (bps)	1 (189)	2 (162)	3 (134)	4 (54)	1 (125)	2 (106)	3 (68)	4 (37)
	Percent							
19 (192)	3	2			7		1	
20 (194)	74	72	73	69	39	39	40	
21 (196)	10	14	10	17	21	30	21	95[a]
22 (198)	1				1			
23 (200)		1	1		5	1	1	
24 (202)	3	2	1	4	9	1		
25 (204)	7	9	10	7	17	27	37	5
26 (206)	2	2	3	4	2	1		

* 1. New York [Zhong et al., 1994b]; 2. French [Oudet et al., 1993a]; 3. Belgian-Dutch [Buyle et al., 1993]; 4. Finnish [This study].
[a] Comparing the Finnish with each of the other series gave results of χ^2, $P << 0.0001$.

TABLE II. Allele Frequencies of *FRAXAC1* in Four Populations: Controls vs. FraX*

	Control (%)			Fragile X (%)		
CA repeats	1 (n = 189)	2 (n = 130)	3 (n = 54)	1 (n = 125)	2 (n = 73)	3 (n = 37)
17	2	1				
18	22	16	28	34	27	11
19	67	78	61	34	38	84[a]
20	6		2	3	5	
21	4	5	9	29	27	5

* 1. New York [Zhong et al., 1994b]; 2. English [Hirst et al., 1993]; 3. Finnish [This study].
[a] Comparing the Finnish FraX with either the New York or the English FraX series results gave χ^2, $P << 0.001$. However, no significant difference between FraX (84%) and control (61%) in the Finnish sample was present.

mosomes. The birthplaces of the oldest fragile X individuals or carriers in each family that could be identified are shown in Figure 2. The individuals with the most common haplotype, 21-19-16, were found to have come from a broad area surrounding Kuopio. The two with differing FRAXE types came from various places. One control and one fragile X chromosome both had the same related haplotype 21-19-25. A second haplotype (21-18-17), differing from the common fragile X haplotype in both *FRAXAC1* and FRAXE, was found on four (11.1%) fragile X chromosomes but only one (1.9%) control. These individuals were not known to be related but came from a tightly grouped region in the southeast (Fig. 2). A third haplotype (25-21-15) was seen in two fragile X chromosomes (5.6%) but only one (1.9%) control and may represent another recent mutation. The calculated heterozygosity of the three allele haplotype was 92% in the controls but only 42% in the fragile X chromosomes.

Analysis of a New *Alu-L* Polymorphism

There are 14 *Alu* sequences within the *FMR1* gene (GenBank, #L29074). One of these (*Alu-F*) is adjacent to *FRAXAC2* [Zhong et al., 1993]. *Alu-L* is the shortest one and located about 46 kb distal to the *FMR1* CGG repeat. We have determined that *Alu-L* is polymorphic by SSCP analysis (Zhong et al., unpublished data) and found three different types (type A = 85.9%, B = 11.3%, and C = 2.8%) in the New York Caucasian population. The polymorphic *Alu-L* was studied on the background of selected haplotypes in the Finnish controls and fragile X chromosomes. A total of 30 chromosomes (23 controls and 7 fragile X chromosomes) representing 9 different haplotypes were selected for analysis of the *Alu-L* polymorphism; 29 of 30 chromosomes analyzed were of the common type A. No type B or C was observed. However, 1/30 chromosomes had a new type D allele differing from any previously identified. It was found on the common 20-19-16 haplotype background. Overall, *Alu-L* was quite uninformative in the Finnish samples which may reflect the homogenous ethnic background.

TABLE III. Allele Frequencies of FRAXE in the Finnish

GCC repeats	Control (%) (n = 56)	Fragile X (%) (n = 36)
7	1 (1.8)	
12	1 (1.8)	
14	1 (1.8)	
15	11 (19.6)	3 (8.33)
16	17 (30.4)	27 (75.0)[a]
17	5 (8.9)	4 (11.1)
18	2 (3.6)	
19	9 (16.1)	
20	4 (7.1)	1 (2.8)
22	1 (1.8)	
23	1 (1.8)	
24	1 (1.8)	
25	2 (3.6)	1 (2.8)

[a] Difference in modal frequencies between Fragile X and control is significant $\chi^2 = 15.76$, $P < 0.001$

AGG Analysis

The interspersion patterns of AGG within the *FMR1* CGG repeat were analyzed in 55 controls and 8 premutation chromosomes as shown in Table V. Eighteen different alleles were observed. Six had only one AGG at position 9, 10, 11, 12, 13, and 18. The most common position was 10 followed by 9. Eight had two AGGs with the most common positions being 10/19 followed by 9/19. Among the small controls with ≤35 repeats, 66.7% had two AGGs and 33.3% had one AGG. The AGG positions and microsatellite haplotypes for the 8 controls with 36–47 repeats are given in Table VI. The only chromosomes which lacked AGGs were observed in the premutations among which 7 of the 8 lacked AGGs. In both the Finnish and New York samples there was an association with the longer alleles and the alleles with long pure CGG repeats (≥15) of both *FRAXAC1* and *DXS548* as indicated in Table VII.

DISCUSSION

Previous studies of fragile X founder effects in Finland have used markers *DXS548* and *FRAXAC2* [Oudet et al., 1993b; Haataja et al., 1994]. Since we believe *FRAXAC2* maybe unreliable for founder studies, we undertook this analysis using *DXS548* along with three other markers: *FRAXAC1*, FRAXE, and *Alu-L*. *Alu-L* was not useful for this analysis as it had a very low polymorphism frequency in the Finnish, although

TABLE IV. Haplotypes Frequencies of *DXS548-FRAXAC1*-FRAXE in the Finnish

Haplotype *DXS548-FRAXAC1*-FRAXE	Controls (%) (n = 54)	Fragile X (%) (n = 36)
20-18-16	4 (7.4)	
-18-18	1 (1.9)	
-18-19	2 (3.7)	
-19- 7	1 (1.9)	
-19-12	1 (1.9)	
-19-15	9 (16.7)	
-19-16	9 (16.7)	
-19-17	3 (5.6)	
-19-18	1 (1.9)	
-19-19	5 (9.3)	
-19-20	1 (1.9)	
21-18-14	1 (1.9)	
-18-15	1 (1.9)	
-18-16	3 (5.6)	
-18-17	1 (1.9)	4 (11.1)
-18-20	1 (1.9)	–
-19-15	–	1 (2.8)
-19-16	–	27 (75.0)
-19-20	–	1 (2.8)
-19-23	1 (1.9)	–
-19-25	1 (1.9)	1 (2.8)
23-20-16	1 (1.9)	
24-21-20	1 (1.9)	
25-18-20	1 (1.9)	
-21-15	1 (1.9)	2 (5.6)
-21-17	1 (1.9)	
-21-25	1 (1.9)	
26-19-19	1 (1.9)	
-21-22	1 (1.9)	

it did show one variant allele not previously seen. Therefore haplotypes of the three markers *DXS548-FRAXAC1*-FRAXE were used for this analysis. Four haplotypes, differing only in FRAXE, accounted for 83.4% of the Finnish fragile X chromosomes but were rarely found in the controls. We found that 75% of fragile X chromosomes had haplotype 21-19-16. Therefore, this common haplotype is likely to represent the original one upon which a mutation or founder chromosome arose. Three minor alleles, 21-19-15 and 21-19-20, and 21-19-25, observed in individual chromosomes are also likely the result of microsatellite slippage or recombination. Thus, a clear founder effect was present for this set of markers in the Finnish.

The haplotype 21-18-17 was seen in four (11.1%) fragile X chromosomes but only one (1.9%) control. It is likely to represent a different founder and possibly a much more recent new mutation. It could also reflect admixture from an outside founder as the four families identified with this type came from a very localized region (Fig 2). The third haplotype 25-21-15 was seen in two (5.6%) fragile X chromosomes but only one (1.9%) control is also likely to represent an independent founder or new mutation. This is a type more commonly seen in previous Caucasian populations [Zhong et al., 1995] and because the two families with this type were quite separated may possibly reflect recent admixture of immigrants.

In our previous study, we have shown that the AGG interruptions within the pure CGG repeat region of *FMR1* are associated with stability of inheritance and conversely a long pure repeat region is associated with instability [Zhong et al., 1995]. Comparing the Finnish AGG interspersion patterns to our previous study and to other Caucasian populations [Eichler et al., 1994; Hirst et al., 1994; Kunst and Warren, 1994; Snow et al., 1994; Zhong et al., 1995], the distribution of alleles with one or two AGG interspersions in controls is similar. However, none of the Finnish control alleles were seen to have an absence of AGG, whereas in the other population controls, the prevalence is about 6% [Zhong et al., 1995]. Analysis of the AGG interspersions (Table V) showed that the frequency of gray zone

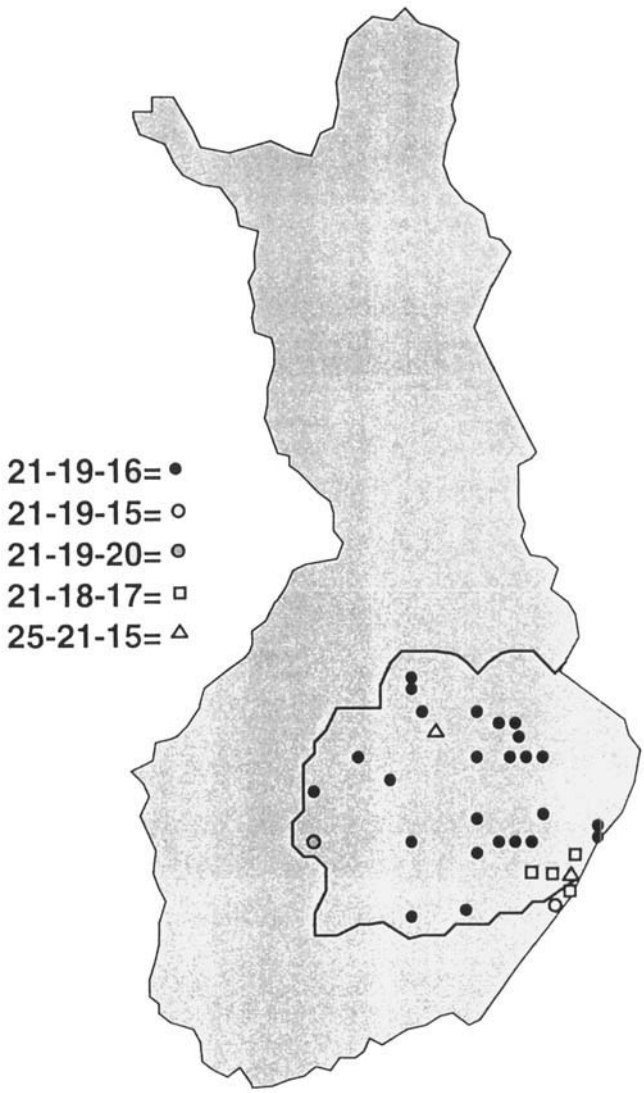

Fig. 2. The birthplaces of the oldest fragile X mutation carriers that could be identified in each unrelated family are shown in this map of Finland. The district around Kuopio, as outlined, is where the majority of samples were obtained. The symbols for the haplotypes are indicated on the left.

TABLE V. Distribution of AGGs Within the *FMR1* CGG Repeats*

FMR1 repeat length	AGG Positions	Number	Percentage
≤35			
23,33	9	2	1.04
23-31	9/19	11	**22.92**
34	9/20	1	2.08
33	9/21	1	2.08
23-34	10	6	**12.5**
23-33	10/19	15	**31.25**
30	10/20	1	2.08
20,23,30	11	3	6.25
23,31	11/17	2	4.16
23	11/20	1	2.08
23,30	12	2	4.16
33	13	1	2.08
26,29	18	2	4.16
36-47			
47	9	1	14.28
36	9/19	1	14.28
47,47,47	10	3	**42.86**
38	10/19	1	14.28
38	10/21	1	14.28
59-155			
59,65,70,75 75,85,155	None	7	**87.5**
70	9	1	12.5

* The most common types are indicated in bold.

alleles that carry two AGGs is highest in the controls with <35 repeats (31/46), is intermediate in gray zone alleles (3/7), and lowest among fragile X premutation chromosomes (0/8). The finding that among the Finnish fragile X premutation chromosomes, 87.5% (7/8) had no AGG (Table V), supports the hypothesis

TABLE VI. Haplotypes of Finnish Grey Zone and Premutation *FMR1* Alleles

FMR1 repeats	AGG positions	Haplotypes (*DXS548-FRAXAC1*-FRAXE)
Grey zone alleles		
36	9/19	25-21-17
38	10/21	20-19-16
38	10/19	20-19-20
38	9	21-19-23[a]
47	10	21-18-14
47	10	25-21-15
47	9	25-21-25
47	10	26-21-22
Premutation alleles		
59	None	20-19-16
65	None	21-19-16[a]
70	None	21-19-16[a]
70	9	26-21-15
75	None	20-19-16
75	None	21-19-16[a]
85	None	20-18-17
155	None	21-19-16[a]

[a] Chromosomes having the 21-19 (*DXS548-FRAXAC1*) type common to fragile X.

TABLE VII. Association of Long Pure Repeats (≥15 CGGs) and Large Microsatellite Alleles in New York and Finland

CGG repeat alleles	Microsatellites			
	FRAXAC1 (20-21 repeats)		*DXS548* (24-26 repeats)	
	Percent			
	NY	F	NY	F
<35 Repeats and <15 Pure CGGs	6	8	8	8
<35 Repeats and ≥15 Pure CGG	17	0	29	18
35–52 Repeats and ≥15 Pure CGG	73	57	55	57
56–180 Repeats	43	12	30	12

that unstable expansion results from the loss of AGG interspersions. Overall, these results support the hypothesis that it is the loss of interspersed AGGs that leads to the fragile X mutation.

In agreement with our previous study [Zhong et al., 1994b], the larger sized *FMR1* CGG repeat chromosomes in the Finnish were also associated with the larger sizes of the adjacent microsatellites (Table VI). Fully 50% (4/8) of the "gray zone" alleles (35–56) were associated with the larger 24-26 CA repeats of *DXS548* and the 21 CA repeat allele of *FRAXAC1* (Table VI), which was a type seen in only 2.2% (1/46) of the smaller controls. Further, in both the New York and Finnish samples there exists an association of longer microsatellite alleles and long pure CGG repeats (Table VI). These results add support to our hypothesis [Zhong et al., 1994b, Brown et al., 1996] that the fragile X mutation is associated with not only linkage disequilibrium, but also with some other genetic effect leading to increased microsatellite allele size or microsatellite instability.

In summary, the fragile X founder effects shown in this study suggest one predominant ancestral mutation accounts for 83.4% of the current mutations in Eastern Finland. Two other less frequent mutations on different microsatellite backgrounds account for 11% and 5.6% and may represent new mutations. Use of the FRAXE repeat was helpful in haplotype analysis even though recombination between the *FMR1* repeat and the FRAXE repeat (or instability of FRAXE itself) was relatively common. Assuming the prevalence of the founder haplotypes corresponds to their antiquity within the population, we postulate that the two rarer haplotypes are of more recent origin as illustrated in Figure 3. Finally, the loss of interspersed AGGs appears to be a mechanism underlying the generation of fragile X mutations.

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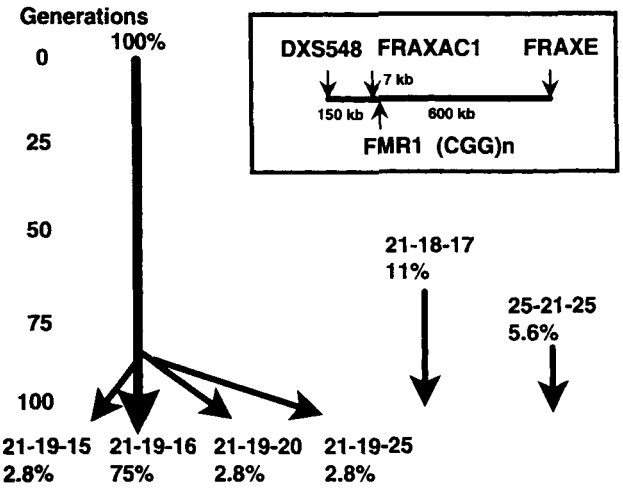

Fig. 3. Three founder haplotypes may reflect the origin of fragile X mutations in eastern Finland. The number of generations may be fewer than 100 and correspond rather to a period of later settlement in the 16th century [Haataja et al., 1994].

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